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RESEARCH ARTICLE

Progeny of *Xenopus laevis* from altitudinal extremes display adaptive physiological performance

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ABSTRACT

Environmental temperature variation generates adaptive phenotypic differentiation in widespread populations. We used a common garden experiment to determine whether offspring with varying parental origins display adaptive phenotypic variation related to different thermal conditions experienced in parental environments. We compared burst swimming performance and critical thermal limits of African clawed frog (*Xenopus laevis*) tadpoles bred from adults captured at high (~2000 m above sea level) and low (~ 5 m above sea level) altitudes. Maternal origin significantly affected swimming performance. Optimal swimming performance temperature (T_{opt}) had a >9°C difference between tadpoles with low altitude maternal origins (pure- and cross-bred, 35.0°C) and high-altitude maternal origins (pure-bred, 25.5°C; cross-bred, 25.9°C). Parental origin significantly affected critical thermal (CT) limits. Pure-bred tadpoles with low-altitude parental origins had higher CT_{max} (37.8±0.8°C) than pure-bred tadpoles with high-altitude parental origins and all cross-bred tadpoles (37.0±0.8 and 37.1±0.8°C). Pure-bred tadpoles with low-altitude parental origins and all cross-bred tadpoles had higher CT_{min} (4.2±0.7 and 4.2±0.7°C) than pure-bred tadpoles with high-altitude parental origins (2.5±0.6°C). Our study shows that the varying thermal physiological traits of *Xenopus laevis* tadpoles are the result of adaptive responses to their parental thermal environments. This study is one of few demonstrating potential intraspecific evolution of critical thermal limits in a vertebrate species. Multi-generation common garden experiments and genetic analyses would be required to further tease apart the relative contribution of plastic and genetic effects to the adaptive phenotypic variation observed in these tadpoles.

KEY WORDS: Anuran, Locomotor performance, Plasticity, Thermal adaptation, Thermal performance curve

INTRODUCTION

Temperature is one of the most important environmental variables impacting all forms of biological rates, functions and ultimately organismal physiology (Fouquette, 1980; Navas, 1996; Navas and Araujo, 2000; Samietz et al., 2005; Angilletta, 2009). Ectothermic species regulate their body temperatures mostly through heat exchange with their environment and, consequently, ectotherms are particularly susceptible to the physiological effects of environmental temperature change (Cossins and Bowler, 1987; Navas and Araujo, 2000). The capacity of ectotherms for physiological performance at various temperatures can be illustrated by a thermal performance curve, where physiological performance is a function of environmental temperature (Huey and Stevenson, 1979; Angilletta, 2009). Maximum performance occurs at an optimum temperature (thermal optimum or T_{opt}) within a performance breadth, while critical temperatures (CT_{max} and CT_{min}) define the limits of an ectotherm's performance, where an animal becomes ecologically or behaviourally dead (Cowles and Bogert, 2006; Deutsch et al., 2008; Angilletta, 2009; Lachenicht et al., 2010). Although some performance measures show great variability, others, such as critical thermal limits, are conservative between populations and even species (Angilletta, 2009; Hoffmann et al., 2013; Bodensteiner et al., 2020; Taylor et al., 2020). Ectotherms may be exposed to daily and seasonal environmental temperature variation close to their thermal limits of performance (Angilletta et al., 2002b; Martin and Huey, 2008). Any such temperature variation could potentially prompt plastic and/or genetic shifts of thermal performance curves (Angilletta, 2009; Logan et al., 2018). Therefore, environmental temperature is an important factor shaping physiological evolution in ectotherms.

The potential of populations to respond to environmental temperature change is critical for species survival. Within an individual's lifetime, ectotherms rely on phenotypically plastic responses to maintain physiological function in the face of environmental temperature variation (Huey et al., 1999; Angilletta, 2009; Seebacher and Franklin, 2011). For example, tadpoles of the African clawed frog, *Xenopus laevis*, exposed to cool temperatures for 4 weeks have greater maximum swimming speeds at cooler test temperatures and lower swimming speeds at higher test temperatures when compared with tadpoles from the same clutches exposed to warmer temperatures (Wilson et al., 2000). Plastic modifications of physiological performance can occur either as a temporary response to short exposures of temperature variation (reversible acclimation) or as an irreversible response to the thermal environment experienced during early development (irreversible acclimation or developmental plasticity) (Wilson and Franklin, 1999; Wilson et al., 2000; Angilletta et al., 2002b; Lagerspetz and Vainio, 2006; Angilletta, 2009). On the scale of multiple generations, performance can evolve in response to the thermal environment. If populations possess sufficient heritable variation in performance variables and there exists a positive relationship between altered performance and fitness, natural selection should favour genetic shifts (i.e. evolution) of thermal performance curves that match environmental temperatures (Angilletta et al., 2002b). However, transgenerational plasticity, such as maternal effects, can produce population variation similar to genetic adaptation (Kawecki and Ebert, 2004). Within maternal effects, phenotypic change is not only determined by the individual's environment (e.g. thermal acclimation) and its genotype (e.g. genetic adaptation), but by the

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phenotype and/or the environment of an offspring's mother. Similarly, paternal effects (the influence of fathers on the phenotypes of offspring via non-genetic mechanisms) have recently been recognised as another important plastic mechanism that can produce a broad range of phenotypic traits across populations (Curley et al., 2011; Rando, 2012; Soubry et al., 2014; Crean and Bonduriansky, 2014). Although it is difficult to design experiments that distinguish between genetic and plastic phenotypic responses, common garden experiments can be used to remove plasticity driven by environmental developmental conditions (i.e. acclimation), and, additionally, cross-breeding of parental types can help distinguish between potential maternal and paternal effects.

Many studies have used common garden experiments as an approach that gives insight into whether observed phenotypic variation in populations is adaptive, although this approach is unable to disentangle epigenetic effects (Merilä and Hendry, 2014; Richter-Boix et al., 2015; Levis and Pfennig, 2016; Sparks et al., 2017; Kosmala et al., 2018; Bodensteiner et al., 2020). Briefly, in order to isolate adaptive contributions to trait differences and remove plastic contributions driven by environmental conditions during development, individuals of known backgrounds are reared/raised/grown under common laboratory or field conditions (Conover and Schultz, 1995; Blanquart et al., 2013). By rearing individuals with pure-bred and crossed/mixed backgrounds in a common environment it is possible to elucidate the contribution of some plastic (e.g. thermal acclimation and maternal effects) and genetic effects to a physiological trait of interest. However, common garden experiments are only logistically feasible with a few organisms, such as some fish and amphibians, given the difficulty in maintaining animals in a limited space for longer periods.

For organisms with complex life histories, such as amphibians, environmental temperature variation can prompt varying thermal responses in different developmental stages (Sherman and Levitis, 2003; Corn, 2005; Ragland and Kingsolver, 2008; Angilletta, 2009; Li et al., 2013; Turriago et al., 2015). Previous studies, for example, indicate that terrestrial adult amphibians have limited acclimation capacities, while the ability to acclimate is present in aquatic amphibian larvae (Wilson and Franklin, 1999; Wilson et al., 2000; Měráková and Gvoždík, 2009; Bartheld et al., 2017; but see Niehaus et al., 2011; Kern et al., 2015). Thermal responses are probably more common in aquatic larvae due to stable environmental cues, i.e. lower temperature variability, in aquatic habitats (Scheiner, 1993; Johnston and Temple, 2002). Furthermore, amphibian larvae are extremely sensitive to alterations in temperature (Shi, 2000) and responses to environmental temperature variation have especially large effects on tadpole growth, development and physiological performance (Skelly and Freidenburg, 2000; Melvin, 2016). Amphibian larvae are also easy to maintain under experimental conditions and in large numbers (Nieuwkoop and Faber, 1956). Tadpoles are, therefore, suitable subjects for studying the evolutionary potential of physiological responses to environmental temperature variation.

Xenopus laevis is a principally aquatic amphibian that normally inhabits permanent water bodies (Measey, 2016). In its native range, *X. laevis* exploits aquatic habitats over diverse altitudinal climatic gradients from sea level to more than 3000 m above sea level (Measey, 2004). Individuals from contrasting altitudes could, therefore, exhibit adaptive physiological responses to their respective thermal environments. *Xenopus laevis* is also a global model organism for various biological studies due to ease of laboratory husbandry and breeding using hormonal stimulants (Nieuwkoop and Faber, 1956; Gurdon and Hopwood, 2003). This makes *X. laevis* a suitable vertebrate study species to characterise potential adaptive physiological phenotypes in a common garden environment.

In this study, we used a common garden experimental approach to determine whether *X. laevis* tadpoles exhibit thermal adaptation corresponding to their parental environments. *Xenopus laevis* adults from contrasting thermal regimes (Fig. 1) and altitudes (∼5 and ∼2000 m above sea level) were bred to produce tadpoles with different parental thermal histories whose critical thermal limits were determined together with burst swimming performance at a range of five test temperatures. Adults were bred to produce both pure- and cross-bred tadpoles with varying maternal and paternal combinations. The study aimed to determine whether offspring with varying parental origins display different levels of thermal responses in performance and critical thermal limits due to different thermal conditions experienced in parental environments.

MATERIALS AND METHODS

Sampling locations

Xenopus laevis (Daudin 1802) adults were collected from two sampling areas in KwaZulu-Natal (KZN), South Africa, in April 2018. The KZN landscape ranges from alpine climates of the Drakensberg escarpment up to ∼3000 m above sea level (annual mean temperature: 9.8°C) to subtropical climates in the east along the coast of the Indian Ocean down to sea level (annual mean temperature: 20.7°C) (Bassa et al., 2016). Collections were made in two areas: the Royal Natal National Park (∼2000 m above sea level), hereafter 'high', and iSimangaliso Wetland Park (∼5 m above sea level), hereafter 'low' (Fig. 1). Funnel traps were baited with chicken livers and distributed in ponds to capture adult *X. laevis*. From each sampling area (high and low), 10 individuals (five males and five females) were obtained from two and three collection sites, respectively. In total, 20 individuals were collected from both sampling areas.

Permits

Ethical clearance for research on captive frogs was obtained from Stellenbosch University Animal Ethics Committee (protocol number 1535); collections in iSimangaliso Wetland Park and the Royal Natal National Park were made with permission from KZN Wildlife (collection permit: OP 3169/2018; import permit: CN28-31-5262).

Adult collection and housing

Captured adults were transported to Stellenbosch University. All individuals were PIT-tagged, following Donnelly et al. (1994), allowing unambiguous identification of individuals. Four to five individuals of the same sex and similar size were maintained in 300×225×240 mm aquaria. All individuals were exposed to a 12 h:12 h light:dark photoperiod at room temperature (20.0°C). Aquaria included PVC pipes that served as refuges for individuals. Adults were fed approximately 3 g of chicken hearts twice a week.

Adult breeding

All breeding took place during May and July 2018. *Xenopus laevis* males and females from high and low altitudes were bred in four parental origin combinations: female (high)×male (high), female (low)×male (low), female (high)×male (low) and female (low)×male (high) (Table 1). Four treatment groups of *X. laevis* offspring with different parental thermal histories were thus generated. Breeding pairs consisting of individuals exclusively from either high or low altitudes were replicated threefold, whilst

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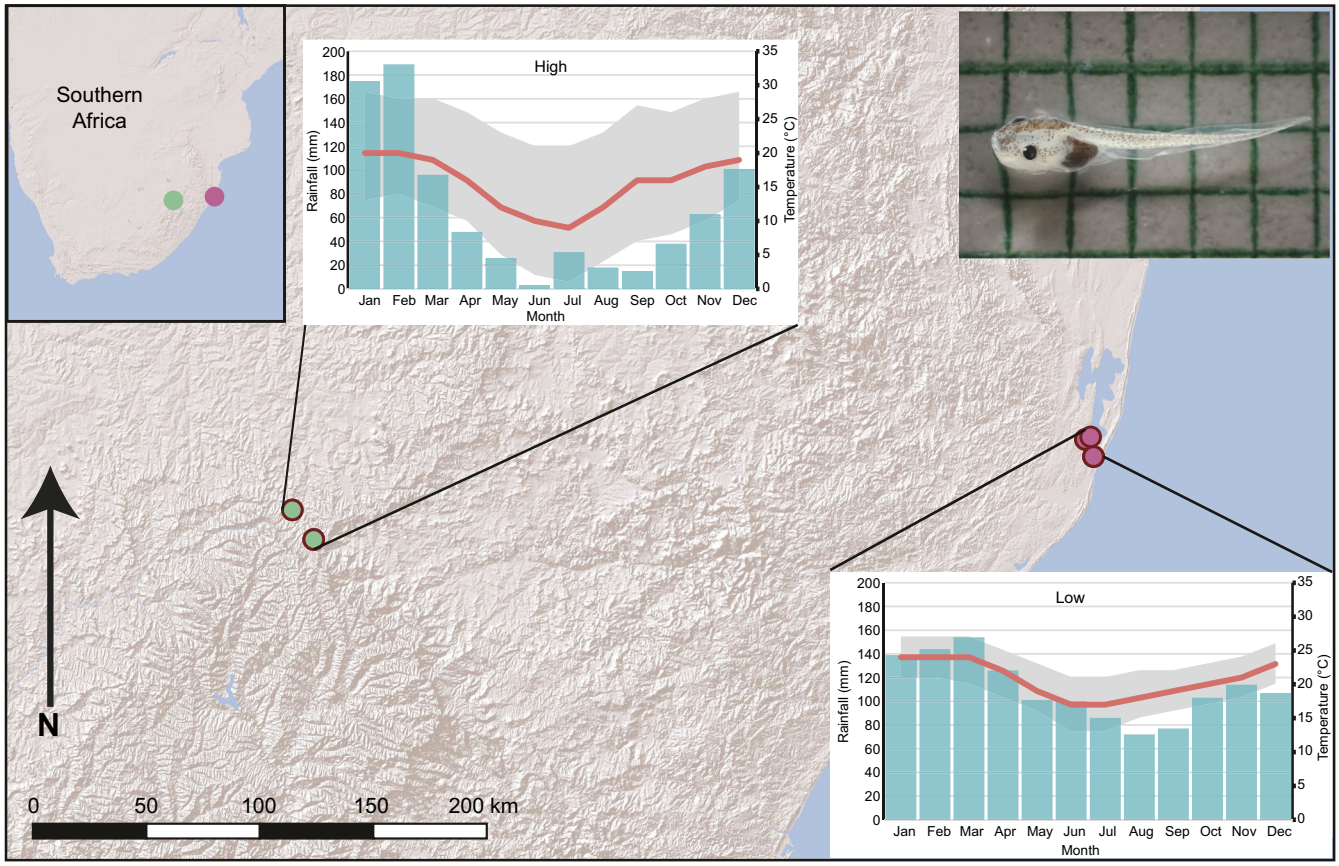

Fig. 1. Location of sampling areas, from high and low altitudes, for *Xenopus laevis* adults in southern Africa. Climatic graphs for low altitude sites (pink: LowA, LowB and LowC) and high altitude sites (green: HighA and HighB) indicate monthly rainfall (blue bars) and temperature (red line). Varying combinations of adults with different origins were bred in a common garden environment to produce stage 46 tadpoles (photo inset: squares, 5 mm).

crossed breeding pairs were replicated twofold. Each adult was only used once for each breeding pair replication. Subsequently, each pair underwent breeding twice to produce two clutches for each breeding pair. Tadpoles from both clutches were selected for critical thermal limit experimental trials, while tadpoles from only one of the clutches from each breeding pair was selected for burst swimming performance trials.

Table 1. Summary of combinations of *Xenopus laevis* adult breeding pairs from different sites (at low and high altitude areas) used to produce 10 tadpole clutches for testing variation of burst swimming performance and critical thermal limit attributed to parental altitude origin

	Parental female origin details			Parental male origin details	
Clutch number	Site	Altitude (masl)		Site	Altitude (masl)
1	LowA	11	×	LowA	11
2	LowB	17	×	LowB	17
3	LowC	32	×	LowC	32
4	HighA	1942	×	HighA	1942
5	HighA	1942	×	HighA	1942
6	HighB	1674	×	HighA	1942
7	LowB	17	×	HighA	1942
8	HighB	1674	×	LowA	17
9	LowA	11	×	HighA	1942
10	HighB	1674	×	LowB	11

masl, metres above sea level.

Individual paired breeding was induced using diluted (1:12) Ovitrelle (Merck Serono; 250 µg per 0.5 ml choriogonadotropin alfa) following the protocol of Davies and Freeman (1995). Male *X. laevis* individuals were injected with 250 IU of human chorionic gonadotrophin (HCG) on the first, second and third day of breeding. Female *X. laevis* individuals were injected with 50 and 500 IU of HGC on the second and third day, respectively. Male and female individuals were kept in separate aquaria and only combined on the third day.

Once injected, individual pairs were placed into 300×225×240 mm breeding aquaria. The aquaria were sealed to prevent individuals from escaping, but were well aerated. Plastic mesh was placed at the bottom of each aquarium to reduce predation by adults on eggs at the bottom of the aquaria. Adults were removed and placed inside their maintenance aquaria the following morning after eggs had been laid. Once adults were removed, the total number of eggs was determined and evaluated for viability. All fertilised eggs were left to hatch in the breeding aquaria where water temperature was maintained at 20.0°C. Once eggs developed into free-swimming tadpoles, these were transferred to holding aquaria with similar conditions to the breeding aquaria.

Tadpole rearing and housing

Tadpoles from each clutch were raised at a water temperature of 20.0°C and at a density of 15 tadpoles per litre. Amphibian larvae kept at such high densities are known to have slower growth and developmental rates (Newman, 1987; Tejedo and Reques, 1994). Slow development ensured that differences in tadpole size tested on

subsequent days were minimised. *Xenopus laevis* larvae were exposed to a 12 h:12 h light:dark photoperiod. For the duration of the rearing period, tadpoles were fed Sera Plankton Tabs (Sera GmbH, Heinsberg, Germany). The tablets were crushed and then mixed with water to form a paste. Small portions of this paste were provided to tadpoles twice a day. Excess quantities of paste were avoided to maintain water quality in aquaria. At approximately 6–7 days of age, when tadpoles reached stage 46 (Nieuwkoop and Faber, 1956), tadpoles with a length of 9–12 mm were selected for burst swimming performance trials.

Burst swimming performance trials

An adaptation of the procedure to evaluate burst swimming performance in response to acute temperature changes, as outlined in Wilson et al. (2000), was applied in this study.

Burst swimming sequences were filmed in a 420×80×170 mm clear plastic experimental test chamber filled with 1 litre of dechlorinated tap water at a depth of 150 mm. Burst swimming performance trials were executed at five test temperatures (means± s.d.): 5.0±0.3, 10.0±0.2, 20.0±0.2, 30.0±0.2 and 35.0±0.3°C. Water temperature was verified with fine copper–constantan (Type K, 36 gauge) thermocouples, connected to a datalogger (TC-08 Thermocouple Datalogger, Pico Technology, St Neots, UK) and calibrated to two decimal place values. The burst swimming performance speed of 10 tadpoles from each breeding pair was assessed at each test temperature. In total, the burst swimming performance speed of 50 tadpoles from each breeding pair was assessed. Each individual was only tested once. To avoid any test sequence or age effects, breeding pairs or treatment groups were assigned to different test orders.

Before the onset of each burst swimming performance trial, selected tadpoles from a breeding pair were placed in a holding aquarium filled with 1 litre of dechlorinated tap water at 20.0°C (developmental water temperature). Water temperature in the holding aquarium and test chamber was cooled or heated at a rate of 0.1°C min^{-1}. Once water temperature of the holding and test chamber reached the desired test temperature, tadpoles were moved from the holding aquarium to the test chamber in 50 ml of water. Each individual tadpole was allowed an equilibration period of at least 10 min inside the test chamber before initiation of burst swimming performance trials. At the onset of a performance sequence *X. laevis* larvae were probed with a fine wire, inducing a startle response. The startle response was filmed as a burst swimming performance sequence. At least five 'startle responses' for each individual were recorded at their respective test temperature.

Performance sequences were filmed using a Canon G16 at 120 frames s^{-1} by recording the image on a mirror. The mirror was held at an angle of 45 deg at the side of the test chamber. A 10×10 mm scale was filmed at the onset of each trial, which was used to scale measurements in pixels to metric units.

Video analysis

High-speed video recordings were reviewed to choose burst swimming performance sequences for computerised motion analysis. As swimming performance was recorded in two dimensions, only tadpoles whose burst swimming sequences were perpendicular to the recording camera were used to accurately analyse swimming performance. After selection of appropriate videos, a minimum of three videos were analysed per tadpole.

Video tracking software (Tracker; http://www.cabrillo.edu/~dbrown/tracker/) was utilised to obtain *x*- and *y*-coordinates of the central point of each tadpole across all frames. Raw coordinates were exported to Excel (Microsoft, Redmond, WA, USA) and the displacement of the individual was calculated for each burst swimming sequence. Raw displacement data were then smoothed using a zero-phase shift low-pass Butterworth filter (Winter, 2004) with the cut-off frequency set at 25 Hz. From the smoothed data the following variables were calculated: maximum burst swimming speed (velocity; U_{max}), maximum acceleration (A_{max}), time taken to achieve maximum velocity ($t_{U_{max}}$), the distance covered during the first 200 ms (D_{200}) and the total distance travelled in one burst swimming sequence (D_{total}).

Critical thermal limits trials

A minimum of 16 *X. laevis* tadpoles at stage 46 (Nieuwkoop and Faber, 1956) from each clutch were used for either critical thermal maximum (CT_{max}) or critical thermal minimum (CT_{min}) trials. Individuals were tested separately in a two-compartment cooling/heating chamber. The circular inner chamber contained 100 ml of dechlorinated tap water at a depth of 95 mm (chamber width 40 mm). This chamber exchanged heat with the outer chamber through which water was pumped at an adjustable rate. The water temperature of the outer chamber was thus manipulated to control the water temperature of the inner chamber.

At the onset of each trial, an individual tadpole was moved from its aquarium (20.0°C) and placed inside the inner compartment at a water temperature of 20.0°C. Prior to heating (CT_{max}) or cooling (CT_{min}), tadpoles were allowed 10 min to adjust to their surroundings. Individuals were exposed to a constant heating/cooling rate of 0.3°C min^{-1} until an endpoint was attained. Temperature and oxygenation differences inside were minimised by gently stirring the water of the inner compartment throughout critical thermal limit trials. To determine the endpoint, the tail of the tadpole was probed gently every 60 s by sliding a thin wire under its tail, after which the tadpole generally righted itself. The point at which the tadpole was unable to right itself after 30 s was defined as the CT_{max} or CT_{min}. Owing to the small size of the tadpoles, it can be assumed that body temperature is equivalent to water temperature (Lutterschmidt and Hutchison, 1997). CT_{max} and CT_{min} were thus recorded as the water temperature beside the tadpole, verified with a thermocouple (Type K, 36 gauge) connected to a datalogger (TC-08 Thermocouple Datalogger).

Statistical analyses

Prior Wilcoxon signed rank tests indicated no significant differences of any performance variables across test dates ($P<0.05$ for all comparisons). General linear mixed-effects models (GLMM) were utilised to determine the effect of parental origin (i.e. thermal history) on burst swimming performance variables. Response variables included U_{max}, A_{max}, $t_{U_{max}}$, D_{200} and D_{total}. Prior to analyses, model assumptions (e.g. normality, homogeneity and independence) were assessed according to Zuur et al. (2010). As data met model assumptions, GLMMs with a Gaussian error distribution were utilised in analyses. Maximum likelihood (ML) was preferred over the default restricted maximum likelihood (REML) method as we intended to compare models with different fixed effect structures. A full model included two fixed factors, temperature (as a linear, second- or third-order polynomial contrast) and parental origin, and their interaction. Parental origin had four levels specifying the original altitudinal origin area of adults (high or low). All models were fitted with random intercepts, collection site (Table 1) and age of tadpoles. Variance of the random effects was minimal, so accounting for variation due to site and age of

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tadpoles was unnecessary (s.d.<1.0). Relative importance of competing models was evaluated using Akaike's information criterion (AIC). To evaluate the variance of data explained by each model, marginal (fixed effects) and conditional (fixed and random effects) R^2 values were calculated according to Nakagawa and Schielzeth (2013) using the 'r.squaredGLMM' function in the package MuMIn (https://cran.r-project.org/package=MuMIn). Additionally, the *F*-statistic and associated *P*-values are reported to examine the effect of fixed effects on the dependent variables. Each thermal performance curve (TPC) was modelled according to best-fit models obtained from GLMM analyses; this allowed the computation of a predicted value for the dependent variable, and an estimated standard error on that value, for each temperature value.

The effect of parental origin (fixed effect) on critical thermal limits was analysed using a GLMM. Pre-processing of models was completed as stated previously. Models were fitted with random intercepts, collection site and age of tadpoles. Variance of the random effects was minimal, so accounting for variation due to site and age of tadpoles was unnecessary (s.d.<2.0).

All GLMMs were fitted using the blmer function from the lme4 and blme package in R (Chung et al., 2013; Bates et al., 2015; https://www.r-project.org/).

RESULTS

Burst swimming performance

The interaction between parental origin and the third-order polynomial term of temperature significantly affected all burst swimming performance variables. Significant interaction effects indicated that the effect temperature on the response variables is different between tadpoles with contrasting parental origins. Pure- and cross-bred tadpoles with high-altitude maternal origins displayed similar burst swimming performance curves (Fig. 2, Fig. S1). Pure- and cross-bred tadpoles with low-altitude maternal origins also displayed similar burst swimming performance curves (Fig. 2, Fig. S1). Therefore, tadpoles with the same maternal origins exhibited similar thermal performance curves.

The interaction between temperature as a third-order polynomial function and parental origin significantly affected maximum burst swimming speed (maximum velocity; U_{max}) of tadpoles (Table 2, Table S1). Most of the variance described by the model was derived from the fixed effect, the interaction between temperature and parental origin (Table 2, Table S1). Based on model estimates, tadpoles with low-altitude maternal origins achieved optimum maximum velocity at a higher temperature than tadpoles bred from high-altitude females (Fig. 2, Table 3). Maximum acceleration was also significantly affected by the interaction between temperature (third-order polynomial function) and parental origin (Table 2, Table S1). A substantial amount of variance was explained by the model's fixed effect, parental origin (Table 2, Table S1). Tadpoles with low-altitude maternal origins attained peak acceleration at a higher temperature compared with tadpoles with high-altitude maternal origins (Fig. S1; Table 3).

The time taken before maximum velocity is obtained ($t_{U_{max}}$) was significantly affected by the interaction between temperature (third-order polynomial function) and parental origin (Table 2, Table S1, Fig. S1). However, the model including this interaction only slightly outcompeted five other models, including the model that contained the third-order polynomial function for temperature as the only predictor of $t_{U_{max}}$. The variance described by the model was also minimal (Table 2, Table S1).

The distance travelled during the first 200 ms of a burst swimming sequence (D_{200}) was also significantly affected by the interaction between the third-order polynomial temperature and parental origin (Table 2, Table S1). Tadpoles bred from low-altitude females swam a greater distance after 200 ms at the highest temperatures and shorter distances at cooler temperatures in comparison with tadpoles with high-altitude maternal origins (Fig. S1, Table 2). Most of the variance described by the model was derived from the fixed effect, the interaction between temperature and parental origin (Table 2, Table S1). Total distance travelled in one burst swimming sequence (D_{total}) was also significantly affected by the interaction between temperature as a third-order polynomial function and parental origin (Table 2, Table S1). However, a small amount of variance was explained by the model including the interaction between temperature and parental origin (Table 2, Table S1). Tadpoles with low-altitude maternal origins travelled greater distances in one burst swimming sequence at high temperatures and shorter distances at lower temperatures, in comparison with tadpoles bred from high-altitude maternal origins (Fig. S1, Table 3).

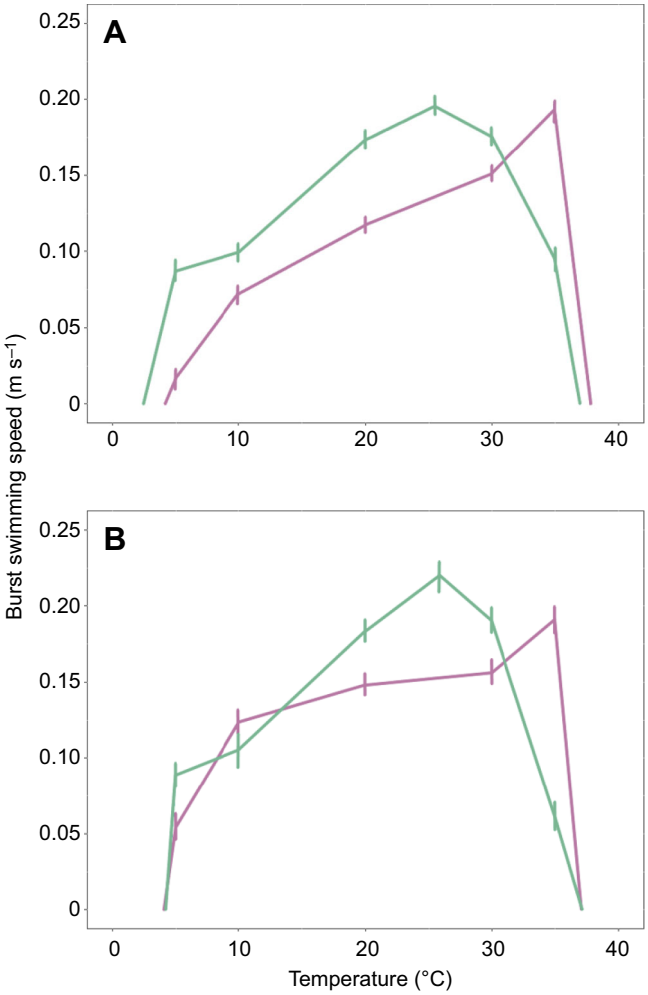

Fig. 2. Maximum burst swimming speed with standard errors at five test temperatures in *X. laevis* tadpoles. (A) High-altitude parental origins (green line, *N*=30 for each test temperature) and low-altitude parental origins (pink line, *N*=30 for each test temperature). (B) Crossed parental origins with high-altitude maternal origins (green line, *N*=20 for each test temperature) and crossed parental origins with low-altitude maternal origins (pink line, *N*=20 for each test temperature). The interaction between parental origin and temperature significantly affected maximum burst swimming speed of tadpoles (GLMM, *P*<0.05).

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Table 2. Summary of best-fit mixed models analysing the effect of *X. laevis* tadpole parental origin on burst swimming speed, maximum acceleration, time taken before maximum velocity is attained, total distance travelled in the first 200 ms and total distance travelled in one swimming sequence

	Explanatory variable								
Dependent variable	Fixed	Random	d.f.	*F*-statistic	AIC	ΔAIC	R^2_m	R^2_c	*P*-value
U_{max}	**Parental origin×temperature**	**Site, age**	**11**	**119.6**	**−1802.8**	**0.0**	**0.6**	**0.7**	**<0.0001**
	Null	Site, age	4		−1351.4	451.4	0.0	0.3	
A_{max}	**Parental origin×temperature**	**Site, age**	**11**	**82.4**	**1797.5**	**0.0**	**0.5**	**0.6**	**<0.0001**
	Null	Site, age	4		2116.7	319.2	0.0	0.2	
$t_{U_{max}}$	**Parental origin×temperature**	**Site, age**	**11**	**3.5**	**1113.5**	**0.0**	**0.1**	**0.2**	**<0.05**
	Null	Site, age	4		1145.3	31.8	0.0	0.1	
D_{200}	**Parental origin×temperature**	**Site, age**	**11**	**53.7**	**−3573.6**	**0.0**	**0.6**	**0.6**	**<0.0001**
	Null	Site, age	4		−3254.3	319.4	0.0	0.2	
D_{total}	**Parental origin×temperature**	**Site, age**	**11**	**12.4**	**−1595.2**	**0.0**	**0.2**	**0.3**	**<0.001**
	Null	Site, age	4		−1537.0	58.2	0.0	0.1	

U_{max}, burst swimming speed (maximum velocity); A_{max}, maximum acceleration; $t_{U_{max}}$, time taken before maximum velocity is attained; D_{200}, total distance travelled in the first 200 ms; D_{total}, total distance travelled in one swimming sequence; Fixed, fixed explanatory variable; Random, random explanatory variable; AIC, Akaike's information criterion; R^2_m, marginal R^2; R^2_c, conditional R^2. For each dependent variable, the selected model, i.e. the one with the lowest AIC, is indicated in bold.

Critical thermal limits

Parental origin had a significant determining effect on the critical thermal limits of tadpoles. For CT_{max}, the model including parental origin of tadpoles only slightly outcompeted the null model (Table 4). Pure-bred tadpoles with high- and low-altitude parental origins exhibited a CT_{max} of 37.0±0.8 and 37.8±0.8°C, respectively. Cross-bred tadpoles with high- and low-altitude maternal origins displayed CT_{max} of 37.1±0.7 and 37.1±0.8°C, respectively, similar to pure-bred high-altitude tadpoles (Fig. 3). Parental origin was also included in the best-fit model significantly predicting the CT_{min} of *X. laevis* tadpoles (Table 4). Tadpole CT_{min} with pure-bred low- and high-altitude parental origins was 4.2±0.7 to 2.5±0.6°C, respectively, and for cross-bred tadpoles with high- and low-altitude maternal origins CT_{min} was 4.2±0.7 and 4.1±0.7°C, respectively, similar to those of pure-bred low-altitude parental origins (Fig. 3).

Table 3. Summary of GLMM analyses of predicted thermal optimum of *X. laevis* tadpoles with varying parental origins for burst swimming performance, maximum acceleration, time taken before maximum velocity is attained, total distance travelled in the first 200 ms and total distance travelled in one swimming sequence

	Parental altitude origin			
Performance variable	Female	Male	Performance	T_{opt} (°C)
U_{max} (m s^{-1})	Low	Low	0.19±0.01	35.0
	Low	High	0.19±0.01	35.0
	High	High	0.20±0.01	25.5
	High	Low	0.22±0.01	25.9
A_{max} (m s^{-2})	Low	Low	6.35±0.25	35.0
	Low	High	5.98±0.34	35.0
	High	High	5.97±0.23	25.9
	High	Low	6.45±0.34	25.9
$t_{U_{max}}$ (s)	Low	Low	0.08±0.35	35.0
	Low	High	0.04±0.24	25.9
	High	High	0.00±0.08	20.9
	High	Low	0.00±0.33	22.8
D_{200} (m)	Low	Low	0.02±0.00	35.0
	Low	High	0.02±0.00	35.0
	High	High	0.02±0.00	24.7
	High	Low	0.03±0.00	24.7
D_{total} (m)	Low	Low	0.10±0.01	35.0
	Low	High	0.06±0.01	25.5
	High	High	0.10±0.00	13.7
	High	Low	0.10±0.01	25.1

Performance values are means±s.e.m. T_{opt}, predicted thermal optimum; U_{max}, burst swimming speed (maximum velocity); A_{max}, maximum acceleration; $t_{U_{max}}$, time taken before maximum velocity is attained; D_{200}, total distance travelled in the first 200 ms; D_{total}, total distance travelled in one swimming sequence.

DISCUSSION

The results of our study indicate that *X. laevis* tadpoles exhibit variation of physiological performance in response to contrasting thermal regimes experienced in parental environments. Population differentiation of physiological variables is widely explored across vertebrates but rarely investigated among amphibian larvae (but see Skelly and Freidenburg, 2000; Orizaola et al., 2010; Hossack et al., 2013; Rudin-Bitterli et al., 2020). Although non-genetic effects such as acclimation could contribute to the observed population differentiation in *X. laevis*, such confounding effects were minimised by maintaining all adults and their offspring under common environmental conditions (Houot et al., 2010; Wu et al., 2018). Tadpoles with identical maternal origins had similar physiological performance variation irrespective of their paternal origin; a pattern that could be indicative of transgenerational plasticity (i.e. maternal effects), mitochondrial DNA or epigenetic effects. Critical thermal limits, however, exhibited contrasting inheritance patterns, with a dominant phenotype for CT_{max} and CT_{min} inherited by cross-bred tadpoles, irrespective of their maternal or paternal origin. These results provide potential evidence for adaptive variation of critical thermal limits in *X. laevis* tadpole populations. Only a few studies have conclusively demonstrated that intraspecific phenotypic change in vertebrate species is the result of adaptive variation (Urban et al., 2014; Merilä and Hendry, 2014; Luquet et al., 2015; Tedeschi et al., 2016; Albecker and McCoy, 2017; Llewelyn et al., 2018). Our study not only indicates that population differentiation in *X. laevis* tadpoles is probably driven by a response to varying parental thermal environments, but also provides potential evidence for local adaptation of tadpole critical thermal limits to environmental extremes.

Large-scale climatic variation should select for adaptation of critical thermal limits in response to climatic extremes in widespread populations (Conover and Schultz, 1995; Ashton, 2004; Blanckenhorn and Demont, 2004), as passing temperatures

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Table 4. Summary of best-fit mixed models analysing the effect of *X. laevis* tadpole parental origin on critical thermal limits (CT_{max} and CT_{min})

	Explanatory variable								
Dependent variable	Fixed	Random	d.f.	*F*-statistic	AIC	ΔAIC	R^2_m	R^2_c	*P*-value
CT_{max}	**Parental origin**	**Site, age**	**5**	**3.3**	**342.0**	**0.0**	**0.1**	**0.6**	**<0.05**
	Null	Site, age	4		340.0	2.0	0.0	0.6	
CT_{min}	**Parental origin**	**Site, age**	**5**	**26.5**	**335.4**	**0.0**	**0.4**	**0.8**	**<0.001**
	Null	Site, age	4		353.6	18.2	0.0	0.9	

Fixed, fixed explanatory variable; Random, random explanatory variable; AIC, Akaike's information criterion; R^2_m, marginal R^2; R^2_c, conditional R^2. For each dependent variable, the selected model, i.e. the one with the lowest AIC, is indicated in bold.

beyond critical thermal limits has very strong and immediate fitness costs (death) (Angilletta, 2009). Even so, most intraspecific studies examining selection and heritability of critical thermal limits suggest that these physiological variables are conserved among populations and even species (Addo-Bediako et al., 2000; Angilletta et al., 2002a; Gvoždík and Castilla, 2001; Moritz et al., 2012; Hoffmann et al., 2013; Araujo et al., 2013; Logan et al., 2014; Pontes-da-Silva et al., 2018; Bodensteiner et al., 2020; Taylor et al., 2020). Our study is one of few demonstrating adaptive variation of critical thermal limits within a species. Pure-bred *X. laevis* tadpoles with low-altitude parental origins had significantly higher CT_{max} and CT_{min} than tadpoles with high-altitude parental origins. Interestingly, all cross-bred tadpoles exhibited CT_{max} similar to pure-bred high-altitude tadpoles and CT_{min} similar to pure-bred low-altitude tadpoles. This pattern of inheritance makes it unlikely that our results are the product of acclimation or transgenerational plastic responses, such as maternal and paternal effects. Instead, a dominant phenotype appears to be inherited by all cross-bred tadpoles: high-altitude CT_{max} and low-altitude CT_{min}, irrespective of maternal or paternal origins. This also suggests that this trait is not inherited via maternally inherited mitochondrial DNA. Therefore, variation of critical thermal limits among *X. laevis* tadpoles could reflect genetic adaptation to altitudinal extremes. Moreover, transgenerational epigenetic effects, such as DNA methylation or histone modification, may produce similar results as observed in this study, as is the case for other common garden studies (Meier et al., 2014; Diamond et al., 2017), although the magnitude of these effects on physiological trait variation has been suggested to be small (Ho and Burggren, 2010; Massamba-N'Siala et al., 2014). We propose that multi-generation common garden experiments combined with evidence for selection from DNA sequence data can help determine the relative contribution of non-genetic and genetic effects to the physiological trait variation observed in these tadpoles.

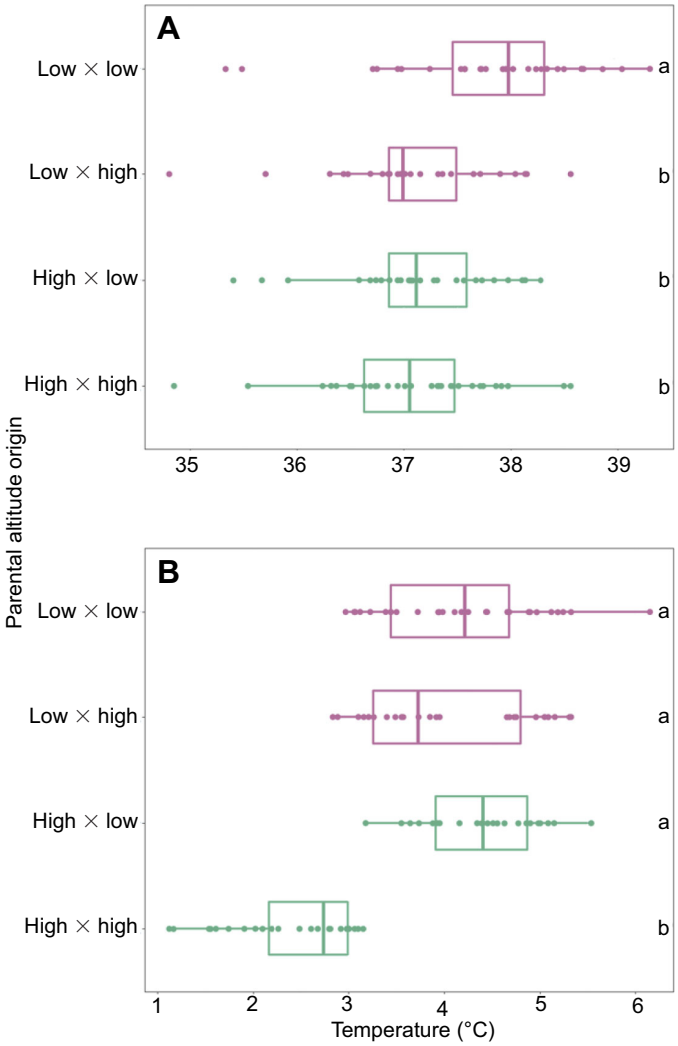

Fig. 3. Critical thermal maxima and minima of *X. laevis* larvae bred from adults with varying thermal histories. (A) Critical thermal maximum; (B) critical thermal minimum. *N*=48 for pure-bred groups and *N*=32 for cross-bred groups. Box plots in pink indicate critical thermal limits of tadpoles bred from low-altitude females, and box plots in green indicate tadpoles bred from high-altitude females. The mid-bold line and whiskers in the box plots represent the medians and range of the lower quartile (25th percentile) and upper quartile (75th percentile). Parental origin determined critical thermal limits of tadpoles (GLMM, $P<0.05$). Different lowercase letters to the right of boxplots indicate significant differences of critical thermal limits.

Tadpoles with distinct parental origins displayed varying physiological responses to environmental extremes through shifts in optimum performance temperatures. Depending on maternal origin, *X. laevis* tadpoles maintained comparably high performance at divergent optimum temperatures. Divergent maximum performance temperatures are a well-known adaptive response to varying thermal regimes in ectotherm populations, although not observed as often in amphibians (Angilletta, 2009; Kolbe et al., 2010; Wu et al., 2018). A previous study demonstrated no shifts of optimal performance temperatures in *X. laevis* adults between two native populations experiencing varying thermal environments (Stellenbosch and St Lucia, South Africa; Araspin et al., 2020). It is possible that in its native range, *X. laevis* displays different thermal responses to environmental change through ontogeny (Sherman and Levitis, 2003; Angilletta, 2009). In laboratory *X. laevis* populations, tadpoles can acclimate swimming performance to surrounding temperatures, while the adults do not demonstrate any acclimation capacity (Wilson et al., 2000). Adaptive genetic or non-genetic responses might be more prominent in amphibian larvae due to their marked sensitivity to environmental temperatures and the commensurate effects on tadpole fitness (Shi, 2000; Skelly and Freidenburg, 2000; Melvin, 2016). Variation of phenotypic trait expression across life stages has been previously observed in invasive populations of *X. laevis*, attributed to trait decoupling

(Kruger et al., 2021). It remains to be tested whether adaptive shifts in tadpole optimum performance temperatures observed in this study are reflected in adult *X. laevis*.

The adaptive capacity of populations to respond to environmental change is crucial for their perseverance. Adaptive phenotypic variation can play an important role in the success and spread of invasive species, such as *X. laevis*, in novel environments (McCann et al., 2014). Adaptive evolution within the native range prior to introduction is often overlooked, despite its important role in invasion establishment and spread (Lee and Gelembiuk, 2008; Hufbauer et al., 2012). Admixture of previously isolated native populations exhibiting extensive population differentiation of phenotypic traits can increase the chances of invasion success by increasing the adaptive variation of exotic populations (Lavergne and Molofsky, 2007; McCann et al., 2014). The African clawed frog is invasive on four continents (Measey et al., 2012), and admixture of two distinct South African phylogeographic lineages of *X. laevis* has been argued to have contributed to the invasion success of this species in France (de Busschere et al., 2016). Compared with other invasive *X. laevis* populations, the invasive French population has undergone the most divergent niche shift (Rödder et al., 2017). Contrastingly, our study shows that admixture between *X. laevis* populations experiencing divergent thermal regimes does not result in an increased adaptive potential, as mixed-bred tadpoles had the narrowest range of thermal tolerance. Instead, potential plastic responses of physiological performance, as observed in this study, could possibly enhance an individual's ability to respond to rapid environmental change.

Our results show that the thermal physiology of *X. laevis* larvae varies across altitudinal extremes. We propose that this variability is the basis of adaptive responses to parental environments. Specifically, our study is one of few demonstrating intraspecific variation of critical thermal limits. Our results emphasise the potential of diverging *X. laevis* populations as a unique source of adaptive variation and its possible contribution to adaptive shifts in response to novel exotic environments. Conducting studies directed towards assessing the relative roles of genetic and plastic effects in producing phenotypic variation should be considered.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: J.M.; Methodology: C.W., N.K., J.M.; Validation: C.W., N.K., J.M.; Formal analysis: C.W.; Data curation: C.W.; Writing - original draft: C.W.; Writing - review & editing: C.W., N.K., J.M.; Visualization: C.W., J.M.; Funding acquisition: J.M.

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Data availability
Data used in this article can be found on Zenodo at doi:10.5281/zenodo.4638555.

Supplementary information
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